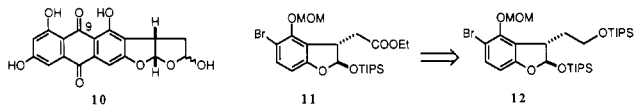
intermediate,[12,13] versicolorin B (**4**) and versicolorin A hemiacetal (**10**), which is reduced to **4** under cell-free conditions, should partition in ^{13}C-labeled form between aflatoxin B_2 (**7**) and B_1 (**9**). Versicolorin A (**5**), however, should incorporate label solely into aflatoxin B_1 (**9**).



10 11 12

Racemic [4'-^{13}C]averufin,[12] [9-^{13}C]versicolorin B (C),[14] [9-^{13}C]versicolorin A hemiacetal, and [9-^{13}C]versicolorin A were adminstered to mycelial suspensions of wild-type *A. parasiticus* (SU-1) under standard conditions.[2,13] After quantification by HPLC analysis of the aflatoxin B_1 and B_2 produced,[15] **7** and **9** were separated and further purified by preparative radial chromatography (silica gel, $CHCl_3$/acetone 20:1). The sites and extent of ^{13}C-incorporation were determined by $^{13}C\{^1H\}$ NMR spectroscopy and mass spectrometry. In each instance the locus of ^{13}C-enrichment in both aflatoxin B_1 and B_2 was that expected. The levels of ^{13}C-incorporation are summarized in Table I.

In *A. parasiticus* (SU-1) about 20 times more aflatoxin B_1 (**9**) is generated than B_2 (**7**). As might be predicted for an earlier pathway intermediate, i.e., averufin (**1**), steady-state flux through the proposed branch point metabolite versicolorin B (**4**) would be expected to lead to equal levels of isotopic labeling in **7** and **9**, as was observed. Addition of versicolorin B (**4**) itself, however, perturbs that steady state and a significantly enhanced enrichment of ^{13}C was observed in the minor metabolite aflatoxin B_2 (**7**). Entirely in keeping with the cell-free experiments above, label from the hemiacetal **10** partitioned similarly to versicolorin B, consistent with its reduction to this tetrahydrobisfuran prior to utilization in the pathway. In contrast, no label from versicolorin A (**5**) was detected in aflatoxin B_2 (**7**) indicating irreversible desaturation of versicolorin B to versicolorin A.

In conclusion, formation of the critical dihydrobisfuran is a three-step process from versiconal acetate (**3**) involving an acetate hydrolysis/cyclization sequence to give versicolorin B (**4**) and an apparent oxidative desaturation step to irreversibly form versicolorin A (**5**). Both 1'-hydroxyversicolorone (**2**) and versiconal acetate (**3**) are isolated as racemates.[16] Cell-free experiments have demonstrated, however, that versiconal acetate (**3**) can be *completely* converted to (-)-versicolorin B (**4**) suggesting that either a single, bifunctional enzyme or two enzymes selectively process one enantiomer of **3** to establish the absolute configuration of the critical bisfuran ring system in **4**. The stereoelectronic arguments made earlier,[17] based on the absolute configuration of averufin (**1**),[18] would give **2** and **3** in the correct configuration for conversion to bisfurans but fail, however, to account for the competing facility of these intermediates to racemize relative to their rates of forward reaction in the biosynthetic pathway. Once formed, separate pathways diverge from versicolorin B (**4**) to give rise to the tetrahydro- and dihydrobisfuran-containing aflatoxins **7** and **9**. The hydroxylated species **10**, which might be imagined to be involved in the oxidation of **4** to **5**, does not undergo dehydration under either cell-free or whole-cell conditions, but is reduced to versicolorin B.[19]

Acknowledgment. We are grateful to the National Institutes of Health for financial support of this research (ES 01670). Major analytical instrumentation used was acquired with funds from the NIH and NSF (NMR: RR01934 and PCM 83-03176; MS: RR 02318).

(19) Hydration, enzymic or chemical, of dihydrobisfurans followed by reduction to the tetrahydro series may provide a minor route for conversion between these bisfuran groups.[3] The results in Table I indicate that this is not a significant process for versicolorin A or for later dihydrobisfuran-containing intermediates in the pathway under the present experimental conditions.

Regioselectivity and Diastereoselectivity in Free-Radical Macrocyclization

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The development of free-radical methods for the synthesis of complex organic molecules continues to attract much attention.[1] Construction of five-membered rings has been one of the cornerstones of recent synthetic developments, but the synthesis of large rings by free-radical cyclization has also recently been reported.[2] Macrocycle yields can be as high as 90% for secondary or tertiary radicals if the alkene is electron deficient. Steric effects have a profound effect on the reaction.[3,4] We report here on new substrates, **1a-d**, for macrocyclization that have X = CH_2 and Z = COOEt or $CONR_2$ (Scheme I). Cyclizations of these compounds are highly selective for the endocyclic product. Furthermore, substrates where Z is a chiral amide derived from alanine, **1c** and **1d**, give products that are highly enriched in one of the four possible diastereomeric cyclic products.

The synthesis of the substrates **1a-d** is straightforward, the key step being phosphonate coupling to form the electrophilic alkene.[5] For compounds **1b-d**, for example, the coupling reaction between $Br(CH_2)_mCOCH_2PO(OEt)_2$[6] and the α dicarbonyl $HCOCONR_2$ gave $Br(CH_2)_mCOCH{=}CHCONR_2$ (70%), which was converted to the iodide by reaction with NaI (quantitative). The substrate **1a** was prepared via the sequence $Br(CH_2)_{12}COCHO$ + $EtOOCCH_2PO(OEt)_2 \rightarrow Br(CH_2)_{12}COCH{=}CHCOEt$ (70%) $\rightarrow$ **1a** (quantitative).

Mixtures of cis and trans geometric isomers were sometimes formed in the coupling reaction, and, if formed, the cis isomers were converted to the trans by reaction with I_2. The dicarbonyl $HCOCONR_2$ was prepared by reaction of the nitrate $O_2NOCH_2CONR_2$ with NaOAc in DMSO;[7] $Br(CH_2)_{12}COCHO$ was prepared by Swern oxidation of $Br(CH_2)_{12}CHOHCH_2OH$.[8] The pyrrolidine used for the amide of **1c** and **1d** was prepared by the method of Schlessinger, and the starting material used in this synthesis was the unnatural (*R*)-alanine since cyclization

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(14) Reduction of **11** with $LiAlH_4$ and protection of the resulting alcohol gave **12**, which was converted in 45% overall yield to versicolorin C (the racemate of versicolorin B) essentially as described for versicolorin A (see preceding paper).

(15) Modification of the method of Schmidt et al. (Schmidt, R.; Mondani, J.; Ziegenhagen, E.; Dose, K. *J. Chromatogr.* **1981**, *207* 435-438).

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Scheme I

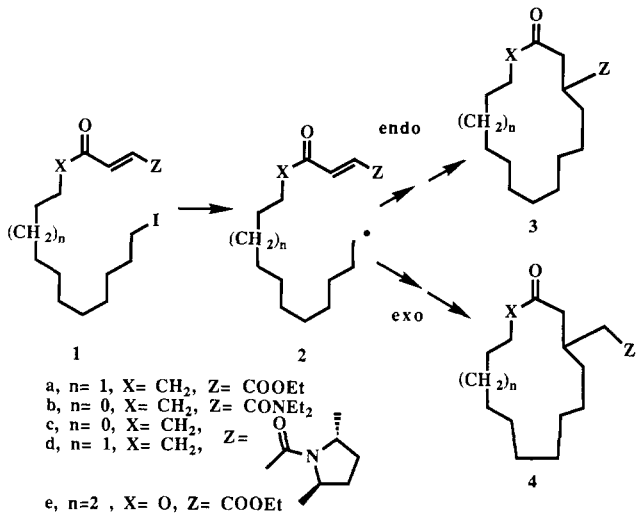


Table I. Product Ratio of Cyclization Products from Iodides **1a–d**[a]

iodide	exo, **4**	endo, **3**
1a	<2 (14)	>98 (15)
1b	1 (13)	10 (14)
	exo 1:exo 2 (13)	endo *R*:endo *S* (14)
1c[b]	1:1	14:1
	exo 1:exo 2 (14)	endo *R*:endo *S* (15)
1d[b]	1:1	13:1
1e[c]	1 (15)	2.5 (16)

[a] Ring size shown in parentheses. [b] Two diastereomeric endocyclic products and two diastereomeric exocyclic products are formed. The product ratio of the four diastereomers is given here. The configuration of the newly formed stereocenter is indicated. [c] Reference 4.

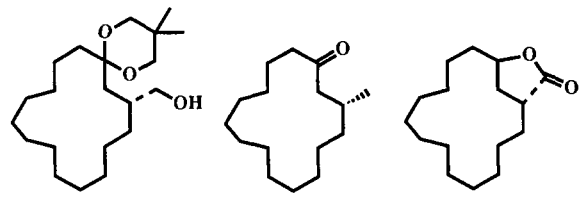
reactions with this auxiliary give natural (−)-(*R*)-muscone for comparison.[9] All new compounds were fully characterized by spectroscopy and elemental analysis.

Cyclization of the primary iodides was carried out under standard tin hydride conditions.[2] In a typical cyclization, described here for **1a**, a 4.5 mM solution of the iodide in refluxing benzene was reacted with 1.1 equiv of Bu_3SnH and 0.1 equiv of the azo initiator AIBN. After 3 h of reaction and purification on silica gel, the mixture yielded 57% cyclic compounds that included endocyclic and exocyclic products. For **1a**, only endocyclic product was isolated, and, if formed, the exocyclic product was present in the product mixture in less than 2%. Endocyclic products could be distinguished from the exocyclic products by mass spectral fragmentation that gives a characteristic loss of CH_2COOR or CH_2CONR_2 from the molecular ion for the exocyclic products.[10] Yields of cyclic products for the amide derivatives **1b–d** (40–45%) are lower than for the ester.

For the pyrrolidine amides **1c** and **1d**, four cyclic products are possible: two exocyclic diastereomers and two endocyclic compounds. All four diastereomers can be separated by HPLC on silica gel (20% ethyl acetate/hexane), and the exocyclic and endocyclic compounds can be readily assigned by mass spectral fragmentation, vide supra. The absolute stereochemistry of the exocyclic compounds **4c** and **4d** has not been assigned, since they are minor products and since they form in a 1:1 ratio. The stereochemistry of the two endocyclic compounds was established by conversion to the natural product muscone as described below. The product distribution of cyclic products formed for the four substrates **1a–d** is presented in Table I.

The regioselectivity and diastereoselectivity shown in the cyclization reactions of the iodides **1a–d** is striking. It seems reasonable to suggest that the balance of polar effects of the ketone vs the ester or amide groups substituting the olefin in **1a–d** promotes endocyclization compared to the fumarate derivative, **1e**,[4] but intermolecular additions of radicals to olefins that are models for the substrates **1c** and **1d** show a 1:1 ratio of regioisomers.[11] The preference for endocyclization in these substrates must therefore arise from the intramolecular nature of these reactions, and substrates where X = CH_2 appear to have an enhanced endocyclization bias compared to those where X = O.[4]

The diastereoselectivity observed in the endocyclization products of cyclizations of **1c** and **1d** can be understood by analysis of the preferred conformation of the amide auxiliary and the vector of approach of the radical in the addition reaction.[11] The preferred conformation of the amide requires that the nucleophilic radical approach the olefin on a vector over the pyrrolidine, thus protecting one face of the olefin from addition. In support of the model, we note that addition to the end of the olefin remote from the amide occurs without diastereoselectivity.



7 (−)-(*R*)-muscone 8

The keto ester **3a**, formed by endocyclization of **1a**, has been converted to racemic muscone. Thus, the ketone was converted to the ketal by reaction with 2,2-dimethylpropanediol, the ester was reduced to the alcohol **7** with lithium aluminum hydride, and **7** was deoxygenated under Barton conditions.[12] The ketal resulting from deoxygenation was deprotected, to give material that was identical with muscone in every respect. The configuration of the newly formed stereocenter in the macrocyclizations of **1c** and **1d** was determined by conversion of the pyrrolidine amide group of **1d** to the methyl group of muscone. The pyrrolidine amide was particularly difficult to hydrolyze or reduce, and only by reduction of the ketone and alcohol-assisted acid hydrolysis could it be removed.[13] The lactone **8** was formed in this sequence, and this lactone was converted to (−)-(*R*)-muscone by standard methodology.[14] A mixture of stereoisomeric alcohols was formed in the borohydride reduction of **3d**, and the mixture of stereoisomers was carried through the sequence. The stereochemistries of products derived from **1c** are assigned by analogy to those from **1d**.

The results of the studies on macrocyclization reported here make it clear that alkene amide auxiliaries provide significant stereoselectivity in radical addition reactions and that the development of models to understand these selectivities is worthwhile. In the accompanying communication, intermolecular reactions are described that are analogous to the intramolecular reactions reported here and a model that accounts for the observed stereoselectivity is presented.

Acknowledgment. This work was supported by grants from the NSF and NIH. N.A.P. gratefully acknowledges the Humboldt Foundation for the award of a Senior Fellowship and Professors B. Giese and R. Polniaszek for helpful discussions.

Supplementary Material Available: Details for the synthesis of **1**, **3**, and **4** (9 pages). Ordering information is given on any current masthead page.

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(14) The conversion of **3d** to (−)-(*R*)-muscone was achieved by the following steps: (a) $NaBH_4$; (b) H_2SO_4/H_2O/dioxane; (c) LAH; (d) *t*-$BuPh_2SiCl$; (e) pyridinium chlorochromate; (f) H^+/2,2-dimethylpropanediol; (g) $Bu_4N^+F^-$; (h) Cl(C=S)OPh; (i) Bu_3SnH; (j) HCl/H_2O. The yield for each step was in excess of 70%, and all products were fully characterized by spectroscopy and elemental analysis or exact mass spectra.